

WHAT IS CLAIMED IS:

1. A method for measuring the activity of a cytochrome P450 enzyme comprising:

- 5 (a) providing a luminogenic molecule wherein the molecule is a cytochrome P450 substrate and a pro-substrate of bioluminescent enzyme;
- (b) contacting the luminogenic molecule with at least one cytochrome P450 enzyme and at least one bioluminescent enzyme to produce a reaction mixture; and
- (c) determining cytochrome P450 activity by measuring luminescence of the reaction mixture.

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2. The method according to claim 1 wherein step (b) further includes a pyrophosphatase.

15 3. The method according to claim 2 wherein the pyrophosphatase is an inorganic pyrophosphatase.

4. The method according to claim 1 wherein the luminogenic molecule, the cytochrome P450 enzyme, and the bioluminescent enzyme are contacted at about the same time.

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5. The method according to claim 1 wherein the luminogenic molecule is contacted with at least one cytochrome P450 enzyme to form a first reaction mixture prior to contacting with the bioluminescent enzyme to form a second reaction mixture.

25 6. The method according to claim 5 wherein the second reaction mixture further comprises a detergent.

7. The method according to claim 6 wherein the detergent is a non-ionic detergent.

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8. A method for measuring cytochrome P450 enzyme activity in a cell comprising:

(a) providing a luminogenic molecule wherein the molecule is a cytochrome P450 substrate and a pro-substrate of bioluminescent enzyme;

(b) contacting a cell with the luminogenic molecule and a bioluminescent enzyme to produce a mixture; and

5 (c) determining cytochrome P450 activity of the cell by measuring luminescence of the mixture.

9. The method according to claim 8 wherein cell expresses the bioluminescent enzyme.

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10. The method according to claim 8 wherein step(b) cell is further contacted with a lysis reagent.

11. The method according to claim 8 wherein the cell is lysed prior to step(b).

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12. The method according to claim 8 wherein the cell is lysed prior to step(c).

13. The method according to claim 8 wherein the cell is contacted first with the luminogenic molecule to produce a first reaction mixture prior to contact with the bioluminescent enzyme to produce a second reaction mixture.

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14. The method according to claim 13 wherein the second reaction mixture further comprises a detergent.

25 15. The method according to claim 14 wherein the detergent is a non-ionic detergent.

16. The method according to claim 13 wherein the second reaction mixture further comprises a pyrophosphatase.

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17. The method according to claim 16 wherein the pyrophosphatase is an inorganic pyrophosphatase.

18. A method for measuring cytochrome P450 enzyme activity in animal tissue comprising:
- (a) providing a luminogenic molecule wherein the molecule is a cytochrome P450 substrate and a pro-substrate of bioluminescent enzyme;
 - 5 (b) contacting an animal tissue with the luminogenic molecule and a bioluminescent enzyme to provide a mixture; and
 - (c) determining cytochrome P450 activity of the tissue by measuring luminescence of the mixture.
- 10 19. The method according to claim 18 wherein the tissue is contacted first with the luminogenic molecule for a first predetermined time period prior to contact with the bioluminescent enzyme to provide a second mixture.
20. The method according to claim 19 wherein the second reaction mixture
15 includes a detergent.
21. The method according to claim 20 wherein the detergent is non-ionic.
22. The method according to claim 20 wherein the second reaction mixture
20 further comprises a pyrophosphatase.
23. The method according to claim 22 wherein the pyrophosphatase is an inorganic pyrophosphatase.
- 25 24. A method for measuring cytochrome P450 enzyme activity in an animal comprising:
- (a) providing a luminogenic molecule wherein the molecule is a cytochrome P450 substrate and a pro-substrate of bioluminescent enzyme;
 - (b) administering the luminogenic molecule to the animal;
 - 30 (c) obtaining a biological sample from the animal; and
 - (d) contacting the biological sample with a bioluminescent enzyme to form a reaction mixture; and

(e) determining cytochrome P450 activity of the animal by measuring luminescence.

25. The method according to claim 24 wherein the reaction mixture further
5 comprises a detergent.

26. The method according to claim 25 wherein the detergent is non-ionic.

27. The method according to claim 24 wherein the reaction mixture further
10 comprises a pyrophosphatase.

28. The method according to claim 27 wherein the pyrophosphatase is an inorganic pyrophosphatase.

15 29. A method for measuring cytochrome P450 enzyme activity in a transgeneic animal having a bioluminescent enzyme transgene, said method comprising:

(a) providing a luminogenic molecule wherein the molecule is a cytochrome P450 substrate and a pro-substrate of bioluminescent enzyme;

(b) administering the luminogenic molecule to a transgeneic animal having a
20 bioluminescent enzyme transgene; and

(c) determining cytochrome P450 activity of the animal by measuring luminescence of tissue from the transgeneic animal.

30. The method according to claim 29 wherein the bioluminescent enzyme
25 transgene is a luciferase transgene.

31. A method for screening a compound for its effect on cytochrome P450 activity comprising:

(a) providing a compound for screening;

30 (b) providing a luminogenic molecule wherein the molecule is a cytochrome P450 substrate and a pro-substrate of bioluminescent enzyme;

(c) contacting the compound, the luminogenic molecule, at least one cytochrome P450 enzyme, and a bioluminescent enzyme to produce a reaction mixture; and

(d) determining cytochrome P450 activity, if any, resulting from the interaction of the compound with the cytochrome P450 enzyme by measuring luminescence of the reaction mixture.

32. The method according claim 31 wherein the compound, luminogenic molecule, the cytochrome P450 enzyme, and the bioluminescent enzyme are contacted at about the same time.

33. The method according claim 31 wherein the compound, luminogenic molecule and at least one cytochrome P450 enzyme are contacted first to form a first reaction mixture prior to contacting with the bioluminescent enzyme to form a second reaction mixture.

34. The method according to claim 33 wherein the second reaction mixture further includes a detergent.

35. The method according to claim 34 wherein the detergent is a non-ionic detergent.

36. The method according to claim 35 wherein step (c) further includes a pyrophosphatase.

37. The method according to claim 36 wherein the pyrophosphatase is an inorganic pyrophosphatase.

38. The method according to claim 31 wherein the compound is contacted first with the one or more cytochrome P450 enzymes to form a first reaction mixture, the first reaction mixture are then contacted with the luminogenic molecule to form a second reaction mixture, and the second reaction mixture is then contacted with a bioluminescent enzyme to form a third reaction mixture.

39. The method according to claim 38 wherein the third reaction mixture further includes a detergent.

5 40. The method according to claim 39 wherein the detergent is a non-ionic detergent.

41. A method for determining the effect of a compound on cytochrome P450 enzyme activity of a cell comprising the steps of:

10 (a) providing a compound for testing (b) contacting a cell with a test compound, a luminogenic molecule and a bioluminescent enzyme, wherein the luminogenic molecule is a cytochrome P450 substrate and a pro-substrate of bioluminescent enzyme; and

 (c) determining cytochrome P450 enzyme activity of the cell, if any, resulting
15 from the exposure of the cell to the test compound by measuring and comparing luminescence from said cell with a second cell not exposed to the test compound.

42. The method according to claim 41 wherein cell expresses the bioluminescent enzyme.
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43. The method according to claim 41 wherein the cell is contacted first with the compound to produce a first reaction mixture prior to contact with the luminogenic molecule to produce a second reaction mixture.

25 44. The method according to claim 43 wherein the second mixture further comprises a bioluminescent enzyme.

45. The method according to claim 43 wherein the bioluminescent enzyme is added to the second reaction mixture after a predetermined time period.
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46. The method according to claim 45 wherein the second reaction mixture further includes a detergent.

47. The method according to claim 46 wherein the detergent is a non-ionic detergent.

48. The method according to claim 41 wherein step (b) further includes a
5 pyrophosphatase.

49. The method according to claim 48 wherein the pyrophosphatase is an inorganic pyrophosphatase.

10 50. A method for determining the effect of a compound on cytochrome P450 enzyme activity of animal tissue comprising the steps of:

- (a) providing a test compound;
- (b) contacting an animal tissue with the test compound, a luminogenic molecule and a bioluminescent enzyme, wherein the luminogenic molecule is a
15 cytochrome P450 substrate and a pro-substrate of bioluminescent enzyme; and
- (c) determining cytochrome P450 enzyme activity of the tissue, if any, resulting from the exposure of the tissue to the test compound by measuring and comparing luminescence from said tissue with a control tissue not exposed to the test compound.

20 51. The method according the claim 50 wherein the animal tissue expresses the bioluminescent enzyme.

52. The method according to claim 50 wherein the tissue is contacted with the
25 test compound to produce a first mixture prior to contact with the luminogenic molecule to produce a second mixture.

53. The method according to claim 52 wherein the second mixture further comprises a bioluminescent enzyme.

30 54. The method according to claim 52 wherein the bioluminescent enzyme is added to the second reaction mixture after a predetermined time period.

55. The method according to claim 54 wherein the second reaction mixture further includes a detergent.

56. The method according to claim 55 wherein the detergent is a non-ionic
5 detergent.

57. The method according to claim 50 wherein step (b) further includes a pyrophosphatase.

10 58. The method according to claim 57 wherein the pyrophosphatase is an inorganic pyrophosphatase.

59. A method for determining the effect of a compound on cytochrome P450 enzyme activity in an animal comprising:

- 15 (a) providing a compound for testing;
(b) administering the test compound to an animal;
(c) administering a luminogenic molecule to the animal, wherein the luminogenic molecule is a cytochrome P450 substrate and a pro-substrate of bioluminescent enzyme;
20 (d) obtaining a biological sample from said animal;
(e) contacting the biological sample with a bioluminescent enzyme; and
(f) determining cytochrome P450 enzyme activity of said animal after exposure of said animal to the test compound by measuring and comparing luminescence from said biological sample with a second biological sample taken from an animal not
25 exposed to said test compound.

60. The method according to claim 59 wherein step (c) is performed after step (b) after a predetermined time period has elapsed.

30 61. The method according to claim 59 wherein the biological sample is taken from the animal just prior to exposure to the test compound.

62. The method according to claim 59 wherein said biological sample comprises blood, serum, bile, urine, feces, or tissue.

63. A method for determining the effect of a compound on cytochrome P450 enzyme activity in an transgeneic animal having a bioluminescent enzyme transgene, said method comprising:
- 5 (a) providing a compound for testing;
- (b) administering the test compound to a transgeneic animal having a bioluminescent enzyme transgene;
- (c) administering a luminogenic molecule to the animal, wherein the luminogenic molecule is a cytochrome P450 substrate and a pro-substrate of the bioluminescent enzyme; and
- 10 (d) determining cytochrome P450 enzyme activity of said animal after exposure of said animal to the test compound by measuring and comparing luminescence from tissue from said transgeneic animal with a second biological sample taken from another transgeneic animal not exposed to said test compound.
- 15
64. The method according to claim 63 wherein step (c) is performed after step (b) after a predetermined time period has elapsed.
65. The method according to claim 63 wherein the bioluminescent enzyme
- 20 transgene is a luciferase transgene.
66. A high throughput method for rapidly screening a plurality of compounds to determine their effect on cytochrome P450 activity, said method comprising:
- (a) providing compounds for screening;
- 25 (b) contacting the compounds to be screened with (i) a luminogenic molecule wherein the luminogenic molecule is a cytochrome P450 substrate and a pro-substrate of bioluminescent enzyme; (ii) one or more cytochrome P450 enzymes; and (iii) one or more bioluminescent enzymes to form reaction mixtures, each reaction mixture having one or more compounds; and
- 30 (c) determining cytochrome P450 enzyme activity, if any, resulting from the interaction of one or more compounds with one or more cytochrome P450 enzymes by measuring luminescence of the reaction mixtures.

67. The method according to claim 66 wherein the compounds are contacted first with the one or more cytochrome P450 enzymes to form first reaction mixtures, the first reaction mixtures are then contacted with the luminogenic molecule to form second reaction mixtures, and the second reaction mixtures are then contacted with a bioluminescent enzyme to form third reaction mixtures.

68. The method according to claim 67 wherein the third reaction mixture further includes a detergent.

69. The method according to claim 68 wherein the detergent is a non-ionic detergent.

70. The method according to claim 66 wherein the compounds are contacted first one or more cytochrome P450 enzymes and the luminogenic molecule to form first reaction mixtures prior to contact with one or more bioluminescent enzymes to form a second reaction mixture.

71. The method according to claim 70 wherein the second reaction mixture further comprises a detergent.

72. The method according to claim 70 wherein the detergent is non-ionic.

73. The method according to claim 66 wherein the compounds are contacted simultaneously or contemporaneously with the one or more cytochrome P450 enzymes and the luminogenic molecule to form first reaction mixtures prior to contacting with one or more bioluminescent enzymes to form second reaction mixtures.

74. The method according to claim 66 wherein step (b) further comprises a pyrophosphatase.

75. The method according to claim 74 wherein the pyrophosphatase is an inorganic pyrophosphatase.

76. A high throughput method for rapidly screening a plurality of compounds to determine their effect on cytochrome P450 activity of a cell, said method comprising:

(a) providing compounds for screening;

5 (b) contacting cells with the compounds to be screened, a luminogenic molecule, and one or more bioluminescent enzymes to form reaction mixtures, wherein the luminogenic molecule is a cytochrome P450 substrate and a pro-substrate of bioluminescent enzyme and each reaction mixture having one or more compounds;

(c) determining cytochrome P450 enzyme activity, if any, resulting from the interaction of one or more compounds with one or more cytochrome P450 enzymes by
10 measuring luminescence of the reaction mixtures.

77. The method according to claim 76 wherein the cells express bioluminescent enzyme.

15 78. The method according to claim 76 wherein bioluminescent enzyme from an exogenous source is used.

79. The method according to claim 76 wherein steps (b) and/or (c) further comprises a pyrophosphatase.
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80. The method according to claim 79 wherein the pyrophosphatase is an inorganic pyrophosphatase.

81. The method according to claim 76 wherein the cells are first contacted
25 with the compounds and luminogenic molecule for a first predetermined time period, then contacted with the bioluminescent enzyme for a second predetermined time period.

82. The method according to claim 81 wherein detergent is present during the second predetermined time period.
30

83. The method according to claim 82 wherein the detergent is a non-ionic detergent.

84. The method according to claim 82 wherein the cells are first contacted with the compounds for a first predetermined time period, then contacted with the luminogenic molecule for a second predetermined time period, then contacted with the bioluminescent enzyme for a third predetermined time period.

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85. The method according to claim 84 wherein detergent is present during the third predetermined time period.

86. The method according to claim 85 wherein the detergent is a non-ionic
10 detergent.

87. The method according to claim 76 wherein the cells are first contacted with the compounds for a first predetermined time period, then contacted with the luminogenic molecule and bioluminescent enzyme for a second predetermined time
15 period.

88. The method according to claim 76 wherein the cells, compounds, luminogenic molecule, and bioluminescent enzyme are contacted simultaneously.
20

89. A high throughput method for rapidly screening a plurality of compounds to determine their effect on cytochrome P450 activity of animal tissue, said method comprising: (a) providing compounds for screening;

(b) contacting animal tissue with the compounds to be screened, a
25 luminogenic molecule, and one or more bioluminescent enzymes to form reaction mixtures, wherein the luminogenic molecule is a cytochrome P450 substrate and a pro-substrate of bioluminescent enzyme and each reaction mixture having one or more compounds;

(c) determining cytochrome P450 enzyme activity, if any, resulting from the
30 interaction of one or more compounds with one or more cytochrome P450 enzymes by measuring luminescence of the reaction mixtures.

90. The method according to claim 89 wherein the tissue expresses at least one bioluminescent enzyme.

91. The method according to claim 89 wherein the tissue is first contacted with the compounds and luminogenic molecule for a first predetermined time period prior to contact with the bioluminescent enzyme.

92. The method according to claim 91, wherein after the first predetermined time period, a detergent is added.

93. The method according to claim 92 wherein detergent and bioluminescent enzyme are added at the same time.

94. The method according to claim 92 wherein detergent is added prior to addition of the bioluminescent enzyme.

95. The method according to claim 89 wherein the tissue is first contacted with the compounds for a first predetermined time period, then contacted with the luminogenic molecule for a second predetermined time period, then contacted with the bioluminescent enzyme for a third predetermined time period.

96. The method according to claim 95, wherein after the second predetermined time period, a detergent is added.

97. The method according to claim 96 wherein detergent and bioluminescent enzyme are added at the same time.

98. The method according to claim 96 wherein detergent is added prior to bioluminescent enzyme.

99. The method according to claim 89 wherein the tissue is first contacted with the compounds for a first predetermined time period, then contacted with the luminogenic molecule and bioluminescent enzyme for a second predetermined time period.

100. The method according to claim 89 wherein the tissue, compounds,
5 luminogenic molecule, and bioluminescent enzyme are contacted simultaneously.

101. The method according to claim 89 wherein steps (b) or (c) further
comprises (iv) a pyrophosphatase.

102. The method according to claim 101 wherein the pyrophosphatase is an
10 inorganic pyrophosphatase.

103. A high throughput method for rapidly screening a plurality of compounds
to determine their effect on cytochrome P450 activity of animal, said method comprising:

- 15 (a) providing compounds for screening;
- (b) contacting a living teleost with the compounds to be screened, a
luminogenic molecule, and a bioluminescent enzyme to form reaction mixtures, wherein
the luminogenic molecule is a cytochrome P450 substrate and a pro-substrate of
bioluminescent enzyme and each reaction mixture having one or more compounds;
- 20 (c) determining cytochrome P450 enzyme activity, if any, resulting from the
interaction of one or more compounds with one or more cytochrome P450 enzymes by
measuring luminescence of the reaction mixtures that include test compounds in
comparison to control mixtures without test compounds. .

25 104. The method according to claim 103 wherein the teleost is transgenic and
expresses bioluminescent enzyme.

105. The method according to claim 103 wherein the teleosts are first contacted
with the compounds and luminogenic molecule for a first predetermined time period prior
30 to contact with the bioluminescent enzyme.

106. The method according to claim 103 wherein the teleosts are first contacted
with the compounds for a first predetermined time period, then contacted with the

luminogenic molecule for a second predetermined time period, then contacted with the bioluminescent enzyme for a third predetermined time period.

107. The method according to claim 103 wherein the telosts are first contacted
5 with the compounds for a first predetermined time period, then contacted with the luminogenic molecule and bioluminescent enzyme for a second predetermined time period.

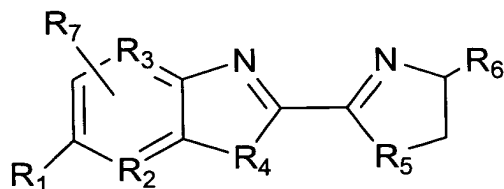
108. The method according to claim 103 wherein the telosts, compounds,
10 luminogenic molecule, and bioluminescent enzyme are contacted simultaneously.

109. The method according to claim 103 wherein steps (b) or (c) further comprises (iv) a pyrophosphatase.

110. The method according to claim 109 wherein the pyrophosphatase is an
15 inorganic pyrophosphatase.

111. The method according to any one of claims 1, 8, 18, 24, 31, 41, 50, 59, 66,
76, 89, and 103 wherein the luminogenic molecule is a luciferin derivative and the
20 bioluminescent enzyme is a luciferase.

112. The method according to claim 111 wherein the luciferin derivative has a formula:



25 wherein

R₁ represents hydrogen, hydroxyl, amino, C₁₋₂₀ alkoxy, substituted C₁₋₂₀ alkoxy, C₂₋₂₀ alkenyloxy, substituted C₂₋₂₀ alkenyloxy, halogenated C₂₋₂₀ alkoxy, substituted halogenated C₂₋₂₀ alkoxy, C₃₋₂₀ alkynyloxy, substituted C₃₋₂₀ alkynyloxy, C₃₋₂₀ cycloalkoxy, substituted C₃₋₂₀ cycloalkoxy, C₃₋₂₀ cycloalkylamino, substituted C₃₋₂₀ cycloalkylamino, C₁₋₂₀ alkylamino, substituted C₁₋₂₀ alkylamino, di C₁₋₂₀ alkylamino,
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substituted diC₁₋₂₀ alkylamino, C₂₋₂₀ alkenylamino, substituted C₂₋₂₀ alkenylamino, di C₂₋₂₀ alkenylamino, substituted di C₂₋₂₀ alkenylamino, C₂₋₂₀ alkenyl C₁₋₂₀ alkylamino, substituted C₂₋₂₀ alkenyl C₁₋₂₀ alkylamino, C₃₋₂₀ alkynylamino, substituted C₃₋₂₀ alkynylamino, di C₃₋₂₀ alkynylamino, substituted di alkylamino, C₃₋₂₀ alkynyl C₂₋₂₀ alkenylamino, or substituted C₃₋₂₀ alkynyl C₂₋₂₀ alkenylamino;

R₂ and R₃ independently represents C or N;

R₄ and R₅ independently represents S, O, NR₈ wherein R₈ represents hydrogen or C₁₋₂₀ alkyl, CR₉R₁₀ wherein R₉ and R₁₀ independently represent H, C₁₋₂₀ alkyl, or fluorine;

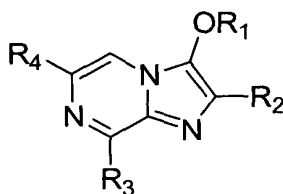
R₆ represents CH₂OH; COR₁₁ wherein R₁₁ represents H, OH, C₁₋₂₀ alkoxide, C₂₋₂₀ alkenyl, or NR₁₂R₁₃ wherein R₁₂ and R₁₃ are independently H or C₁₋₂₀ alkyl; or -OM⁺ wherein M⁺ is an alkali metal or a pharmaceutically acceptable salt; and

R₇ represents H, C₁₋₆ alkyl, C₁₋₂₀ alkenyl, halogen, or C₁₋₆ alkoxide,

with the proviso that R₁ is not OH or NH₂, R₇ is not H, R₆ is not COR₁₁, R₁₁ is not OH, R₃ and R₂ are not both carbon, and R₄ and R₅ are not both S at the same time (luciferin and aminoluciferin).

113. The method according to claims 1, 8, 18, 24, 31, 41, 50, 59, 66, 76, 89 and 103 wherein the luminogenic molecule comprises coelenterazine or coelenterazine derivatives and the bioluminescent enzyme is a luciferase.

114. The method according to claim 113, wherein the coelenterazine derivative has a formula:



wherein

R₁ is C₁₋₂₀ alkyl, branched C₃₋₂₀ alkyl, C₃₋₂₀ cycloalkyl, aralkyl, C₁₋₂₀ alkyl substituted with C₁₋₂₀ alkoxy, hydroxy, halogen, C₁₋₂₀ alkylamino, or diC₁₋₂₀ alkylamino, aralkyl substituted with C₁₋₂₀ alkoxy, hydroxy, halogen, C₁₋₂₀ alkylamino, or diC₁₋₂₀ alkylamino; and

R₂, R₃, and R₄ are independently hydrogen, C₁₋₂₀ alkyl, C₃₋₂₀ cycloalkyl, branched C₃₋₂₀ alkyl, aryl, aralkyl, C₁₋₂₀ alkyl substituted with C₁₋₂₀ alkoxy, hydroxy, halogen, C₁₋₂₀ alkylamino, or diC₁₋₂₀ alkylamino, aralkyl substituted with C₁₋₂₀ alkoxy, hydroxy, halogen, C₁₋₂₀ alkylamino, or diC₁₋₂₀ alkylamino, aryl substituted with C₁₋₂₀ alkoxy, hydroxy, halogen, C₁₋₂₀ alkylamino, or di C₁₋₂₀ alkylamino.

115. The method according to claim 114 wherein R₄ is aryl or aryl substituted with C₁₋₂₀ alkoxy, hydroxy, halogen, C₁₋₂₀ alkylamino, or C₁₋₂₀ dialkylamino.

116. A kit for determining the effect of a substance on cytochrome P450 enzyme activity comprising:

- (a) one or more luminogenic molecules wherein the molecule is a cytochrome P450 enzyme substrate and a pro-substrate of luciferase enzyme; and
- (b) directions for using the kit.

117. The kit according to claim 116, further comprising one or more bioluminescent enzymes.

118. The kit according to claim 110 wherein the bioluminescent enzyme is a luciferase.

119. The kit according to claim 117 wherein the bioluminescent enzyme is a firefly or Renilla luciferase.

120. The kit according to claim 116 further comprising ATP and magnesium ions.

121. The kit according to claim 120 further comprising a detergent.

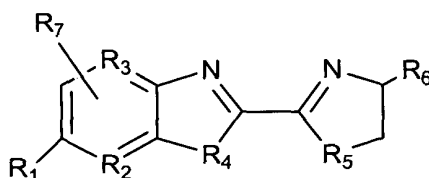
122. The kit according to claim 121 wherein the detergent is non-ionic.

123. The kit according to claim 121 further comprising a pyrophosphatase.

124. The kit according to claim 123 wherein the pyrophosphatase is an inorganic pyrophosphatase.

125. The kit according to claim 116 wherein the luminogenic molecule is a D-luciferin derivative that is a substrate of a cytochrome P450 enzyme and a pro-substrate of a luciferase enzyme.

126. The kit according to claim 125 wherein the luciferin derivative has a formula:



wherein

R₁ represents hydrogen, hydroxyl, amino, C₁₋₂₀ alkoxy, substituted C₁₋₂₀ alkoxy, C₂₋₂₀ alkenyloxy, substituted C₂₋₂₀ alkenyloxy, halogenated C₂₋₂₀ alkoxy, substituted halogenated C₂₋₂₀ alkoxy, C₃₋₂₀ alkynyloxy, substituted C₃₋₂₀ alkynyloxy, C₃₋₂₀ cycloalkoxy, substituted C₃₋₂₀ cycloalkoxy, C₃₋₂₀ cycloalkylamino, substituted C₃₋₂₀ cycloalkylamino, C₁₋₂₀ alkylamino, substituted C₁₋₂₀ alkylamino, di C₁₋₂₀ alkylamino, substituted di C₁₋₂₀ alkylamino, C₂₋₂₀ alkenylamino, substituted C₂₋₂₀ alkenylamino, di C₂₋₂₀ alkenylamino, substituted di C₂₋₂₀ alkenylamino, C₂₋₂₀ alkenyl C₁₋₂₀ alkylamino, substituted C₂₋₂₀ alkenyl C₁₋₂₀ alkylamino, C₃₋₂₀ alkynylamino, substituted C₃₋₂₀ alkynylamino, di C₃₋₂₀ alkynylamino, substituted di C₃₋₂₀ alkynylamino, C₃₋₂₀ alkynyl C₁₋₂₀ alkylamino, substituted C₃₋₂₀ alkynyl C₁₋₂₀ alkylamino, C₃₋₂₀ alkynyl C₂₋₂₀ alkenylamino, or substituted C₃₋₂₀ alkynyl C₂₋₂₀ alkenylamino;

R₂ and R₃ independently represents C or N;

R₄ and R₅ independently represents S, O, NR₈ wherein R₈ represents hydrogen or C₁₋₂₀ alkyl, CR₉R₁₀ wherein R₉ and R₁₀ independently represent H, C₁₋₂₀ alkyl, or fluorine;

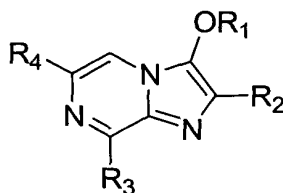
R₆ represents CH₂OH; COR₁₁ wherein R₁₁ represents H, OH, C₁₋₂₀ alkoxide, C₂₋₂₀ alkenyl, or NR₁₂R₁₃ wherein R₁₂ and R₁₃ are independently H or C₁₋₂₀ alkyl; or -OM⁺ wherein M⁺ is an alkali metal or a pharmaceutically acceptable salt; and

R₇ represents H, C₁₋₆ alkyl, C₁₋₂₀ alkenyl, halogen, or C₁₋₆ alkoxide,
with the proviso that R₁ is not OH or NH₂, R₇ is not H, R₆ is not COR₁₁, R₁₁ is not OH, R₃
and R₂ are not both carbon, and R₄ and R₅ are not both S at the same time (luciferin
and aminoluciferin).

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127. The kit according to claim 126 wherein the luminogenic molecule
comprises coelenterazine or a coelenterazine derivative.

128. The kit according to claim 127, wherein the coelenterazine derivative has a
10 formula:



wherein

15 R₁ is C₁₋₂₀ alkyl, branched C₃₋₂₀ alkyl, C₃₋₂₀ cycloalkyl, aralkyl, C₁₋₂₀ alkyl substituted
with C₁₋₂₀ alkoxy, hydroxy, halogen, C₁₋₂₀ alkylamino, or diC₁₋₂₀ alkylamino, aralkyl
substituted with C₁₋₂₀ alkoxy, hydroxy, halogen, C₁₋₂₀ alkylamino, or diC₁₋₂₀
alkylamino; and

20 R₂, R₃, and R₄ are independently hydrogen, C₁₋₂₀ alkyl, C₃₋₂₀ cycloalkyl, branched C₃₋₂₀
alkyl, aryl, aralkyl, C₁₋₂₀ alkyl substituted with C₁₋₂₀ alkoxy, hydroxy, halogen, C₁₋₂₀
alkylamino, or diC₁₋₂₀ alkylamino, aralkyl substituted with C₁₋₂₀ alkoxy, hydroxy,
halogen, C₁₋₂₀ alkylamino, or diC₁₋₂₀ alkylamino, aryl substituted with C₁₋₂₀ alkoxy,
hydroxy, halogen, C₁₋₂₀ alkylamino, or di C₁₋₂₀ alkylamino.

25 129. The kit according to claim 128 wherein R₄ is aryl or aryl substituted with
C₁₋₂₀ alkoxy, hydroxy, halogen, C₁₋₂₀ alkylamino, or C₁₋₂₀ dialkylamino.

130. The kit according to claim 116, further comprising a reversible luciferase
inhibitor.

30

131. The kit according to claim 130, wherein the reversible luciferase inhibitor is 2-(4-aminophenyl)-6-methylbenzothiazole (APMBT) or 2-amino-4-methylbenzothiazole (AMBT).

5 132. A D-luciferin derivative that is a substrate of a cytochrome P450 enzyme and a pro-substrate of luciferase enzyme.

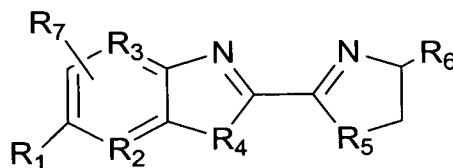
133. A composition comprising the D-luciferin derivative of claim 132.

10 134. The composition of claim 133, further comprising a pyrophosphatase.

135. The composition according to claim 134 wherein the pyrophosphatase is an inorganic pyrophosphatase.

15

135. A D-luciferin derivative having the formula:



20 wherein

R₁ represents hydrogen, hydroxy, C₁₋₂₀ alkoxy or C₁₋₂₀ alkenyloxy wherein the alkoxy and alkenyloxy are substituted with halogen, hydroxy, amino, cyano, azido, heteroaryl or aryl substituted with haloalkyl; or

25 R₁ represents C₃₋₂₀ alkynyloxy; cycloalkoxy, cycloalkylamino, C₁₋₂₀ alkylamino, diC₁₋₂₀ alkylamino, C₂₋₂₀ alkenylamino, diC₂₋₂₀ alkenylamino, C₂₋₂₀ alkenyl C₁₋₂₀alkylamino, C₃₋₂₀ alkynylamino, diC₃₋₂₀ alkynylamino, C₃₋₂₀ alkynyl C₁₋₂₀alkylamino, or C₃₋₂₀ alkynyl C₂₋₂₀alkenylamino, wherein each of the above groups are optionally substituted with halogen, hydroxy, amino, cyano, azido, heteroaryl or aryl substituted with haloalkyl;

30 R₂ and R₃ independently represent C or N;

R₄ and R₅ independently represent S; O; NR₈ wherein R₈ represents hydrogen or C₁₋₂₀ alkyl; CR₉R₁₀ wherein R₉ and R₁₀ independently represent H, C₁₋₂₀ alkyl or fluorine; R₆ represents CH₂OH; COR₁₁ wherein R₁₁ represents hydrogen, hydroxy, C₂₋₂₀ alkenyl, or -OM⁺ wherein M⁺ is an alkali metal or a pharmaceutically acceptable salt; and

5 R₇ represents hydrogen, C₁₋₆ alkyl, C₂₋₂₀ alkenyl, halogen or C₁₋₆ alkoxide, provided that

when R₁ is hydroxy, R₇ is not hydrogen, R₁₁ is not hydroxy, R₂ and R₃ are not both carbon, and R₄ and R₅ are not both S (luciferin);

10 when R₁ is hydrogen, R₇ is not hydrogen, R₁₁ is not hydroxy, R₂ and R₃ are not both carbon, and R₄ and R₅ are not both S (dehydroluciferin); and

when R₁ is hydroxy, R₇ is not hydrogen, R₆ is not CH₂OH, R₂ and R₃ are not both carbon, and R₄ and R₅ are not both S (luciferol).

136. The compound according to claim 135 that is

15 luciferin 6' 2-chloroethyl ether;
luciferin 6' 4-picolinyl ether;
luciferin 6' 4-trifluoromethylbenzyl ether;
luciferin 6' 2-picolinyl ether; or
luciferin 6' 3-picolinyl ether.

20 137. A compound selected from the group consisting of

luciferin 6' 2-chloroethyl ether;
luciferin 6' benzyl ether
luciferin 6' 4-picolinyl ether;
25 luciferin 6' 4-trifluoromethylbenzyl ether;
luciferin 6' phenylethyl ether
luciferin 6' geranyl ether
luciferin 6' prenyl ether
luciferin 6' 2-picolinyl ether; and
30 luciferin 6' 3-picolinyl ether.

138. The compound according to claim 137 selected from the group consisting of

luciferin 6' benzyl ether;
luciferin 6' phenylethyl ether;
luciferin 6' geranyl ether; and
luciferin 6' prenyl ether.

5

139. The compound according to claim 136 selected from the group consisting of

luciferin 6' 2-chloroethyl ether;
luciferin 6' 4-picolinyl ether;
luciferin 6' 4-trifluoromethylbenzyl ether;
luciferin 6' 2-picolinyl ether; and
luciferin 6' 3-picolinyl ether.

10

140. A composition comprising the D-luciferin derivative of claim 135.

15

141. The composition of claim 140, further comprising a pyrophosphatase.

142. The composition according to claim 141 wherein the pyrophosphatase is an inorganic pyrophosphatase.

20

143. A method for measuring P450 enzyme activity comprising

(a) providing a coelentrazine or a coelenterazine derivative that is a P450 substrate and is chemiluminescent;

25

(b) contacting a coelentrazine or coelenterazine derivative with at least one cytochrome P450 enzyme to form a reaction mixture; and

(c) determining cytochrome P450 activity by measuring chemoluminescence of the reaction mixture.

30

144. A method for measuring cytochrome P450 enzyme activity in a cell comprising:

(a) providing a coelentrazine or a coelenterazine derivative that is a P450 substrate and is chemiluminescent;

(b) contacting a cell with the coelenterazine or coelenterazine derivative to form a reaction mixture; and

(c) determining cytochrome P450 activity of the cell by measuring the chemiluminescence of the reaction mixture.

5

145. The method according to claim 144 wherein step (b) cell is further contacted with a lysis agent.

146. The method according to claim 144 wherein the cell is lysed prior to step(b).

10

147. The method according to claim 144 wherein the cell is lyzed prior to step (c).

148. A method for measuring cytochrome P450 enzyme activity in animal tissue comprising:

15

(a) providing coelenterazine or a coelenterazine derivative that is a P450 substrate and is chemiluminescent;

20

(b) contacting an animal tissue with the coelenterazine or a coelenterazine derivative and a bioluminescent enzyme to provide a mixture; and

(c) determining cytochrome P450 activity of the tissue by measuring luminescence of the mixture.

25

149. A method for measuring cytochrome P450 enzyme activity in an animal comprising:

(a) providing coelenterazine or a coelenterazine derivative that is a P450 substrate and is chemiluminescent;

(b) administering the coelenterazine or a coelenterazine derivative to an animal;

(c) obtaining a biological sample from the animal; and

30

(d) determining cytochrome P450 activity of the animal by measuring chemiluminescence of the sample.

150. A method for screening a compound for its effect on cytochrome P450 activity comprising:

- (a) providing a compound for screening;
- (b) providing coelenterazine or a coelenterazine derivative that is a substrate of cytochrome P450 and is chemiluminescent;
- (c) contacting the compound, coelenterazine or a coelenterazine derivative, and a cytochrome P450 enzyme to produce a reaction mixture; and
- (d) determining cytochrome P450 activity, if any, resulting from the interaction of the compound with the cytochrome P450 enzyme by measuring chemiluminescence of the reaction mixture.

151. A method for determining the effect of a compound on cytochrome P450 enzyme activity of a cell comprising the steps of:

- (a) providing a compound for testing;
- (b) contacting a cell with a test compound and coelenterazine or a coelenterazine derivative that is a substrate of cytochrome P450 and that is chemiluminescent; and
- (c) determining cytochrome P450 enzyme activity of the cell, if any, resulting from the exposure of the cell to the test compound by measuring and comparing chemiluminescence from said cell with a second cell not exposed to the test compound.

152. A method for determining the effect of a compound on cytochrome P450 enzyme activity of animal tissue comprising the steps of

- (a) providing a test compound;
- (b) contacting an animal tissue with a test compound and coelenterazine or a coelenterazine derivative that is a substrate of cytochrome P450 and that is chemiluminescent; and
- (c) determining cytochrome P450 enzyme activity of the tissue, if any, resulting from the exposure of the tissue to the test compound by measuring and comparing chemiluminescence from said tissue with a control tissue not exposed to the test compound.

153. A method for determining the effect of a compound on cytochrome P450 enzyme activity in an animal comprising:

- (a) providing a compound for testing;
- (b) administering the test compound to an animal;
- 5 (c) administering coelentrazine or a coelenterazine derivative that is a substrate of cytochrome P450 and that is chemiluminescent;
- (d) obtaining a biological sample from said animal;
- (e) determining cytochrome P450 enzyme activity of said animal after exposure of said animal to the test compound by measuring and comparing
- 10 chemiluminescence from said biological sample with a second biological sample taken from an animal not exposed to said test compound.

154. A high throughput method for rapidly screening a plurality of compounds to determine their effect on cytochrome P450 activity, said method comprising:

- 15 (a) providing compounds for screening;
- (b) contacting the compounds to be screened with (i) coelentrazine or a coelenterazine derivative that is a substrate of cytochrome P450 and that is chemiluminescent; and (ii) one or more cytochrome P450 enzymes, each reaction mixture having one or more compounds; and
- 20 (c) determining cytochrome P450 enzyme activity, if any, resulting from the interaction of one or more compounds with one or more cytochrome P450 enzymes by measuring chemiluminescence of the reaction mixtures.

155. A high throughput method for rapidly screening a plurality of compounds to determine their effect on cytochrome P450 activity of a cell, said method comprising:

- 25 (a) providing compounds for screening;
- (b) contacting cells with the compounds to be screened and coelentrazine or a coelenterazine derivative that is a substrate of cytochrome P450 and that is chemiluminescent to form reaction mixtures, each reaction mixture having one or more
- 30 compounds;
- (c) determining cytochrome P450 enzyme activity, if any, resulting from the interaction of one or more compounds with one or more cytochrome P450 enzymes by measuring chemiluminescence of the reaction mixtures.

156. A high throughput method for rapidly screening a plurality of compounds to determine their effect on cytochrome P450 activity of animal tissue, said method comprising: (a) providing an animal tissue with CYP450 activity

5 (a) providing compounds for screening;

(b) contacting animal tissue with the compounds to be screened and coelenterazine or a coelenterazine derivative that is a substrate of cytochrome P450 and that is chemiluminescent to form reaction mixtures, each reaction mixture having one or more compounds;

10 (c) determining cytochrome P450 enzyme activity, if any, resulting from the interaction of one or more compounds with one or more cytochrome P450 enzymes by measuring chemiluminescence of the reaction mixtures.

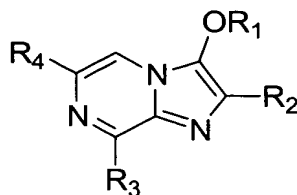
156. A high throughput method for rapidly screening a plurality of compounds to determine their effect on cytochrome P450 activity of animal, said method comprising:

(a) providing compounds for screening;

(b) contacting a living teleost with the compounds to be screened and coelenterazine or a coelenterazine derivative that is a substrate of cytochrome P450 and that is chemiluminescent to form reaction mixtures, each reaction mixture having one or more compounds;

(c) determining cytochrome P450 enzyme activity, if any, resulting from the interaction of one or more compounds with one or more cytochrome P450 enzymes by measuring chemiluminescence of the reaction mixtures that include test compounds in comparison to control mixtures without test compounds. .

158. The method according to any one of claims 143-157 wherein the coelenterazine derivative has a formula:



wherein

R₁ is C₁₋₂₀ alkyl, branched C₃₋₂₀ alkyl, C₃₋₂₀ cycloalkyl, aralkyl, C₁₋₂₀ alkyl substituted with C₁₋₂₀ alkoxy, hydroxy, halogen, C₁₋₂₀ alkylamino, or diC₁₋₂₀ alkylamino, aralkyl substituted with C₁₋₂₀ alkoxy, hydroxy, halogen, C₁₋₂₀ alkylamino, or diC₁₋₂₀ alkylamino; and

5 R₂, R₃, and R₄ are independently hydrogen, C₁₋₂₀ alkyl, C₃₋₂₀ cycloalkyl, branched C₃₋₂₀ alkyl, aryl, aralkyl, C₁₋₂₀ alkyl substituted with C₁₋₂₀ alkoxy, hydroxy, halogen, C₁₋₂₀ alkylamino, or diC₁₋₂₀ alkylamino, aralkyl substituted with C₁₋₂₀ alkoxy, hydroxy, halogen, C₁₋₂₀ alkylamino, or diC₁₋₂₀ alkylamino, aryl substituted with C₁₋₂₀ alkoxy, hydroxy, halogen, C₁₋₂₀ alkylamino, or di C₁₋₂₀ alkylamino.

10

159. The method according to claim 158 wherein R₄ is aryl or aryl substituted with C₁₋₂₀ alkoxy, hydroxy, halogen, C₁₋₂₀ alkylamino, or C₁₋₂₀ dialkylamino.

15 160. The method according to claim 158 wherein the coelentraine derivative is coelenterazine HH, methoxycoelenterazine HH or coelenterazine.

161. A method for enhancing the stability of a luminescent signal generated by a luciferase-based reaction mixture comprising contacting a luciferase with a reversible
20 luciferase inhibitor in an amount effective to enhance the stability and prolong the lifetime of the luminescent signal relative to the luminescent signal generated in a comparable luciferase-based reaction mixture in the absence of the inhibitor.

162. The method according to claim 161 wherein the reversible luciferase
25 inhibitor is a competitive inhibitor.

163. The method according to claim 161 wherein the reversible luciferase inhibitor comprises 2-(4-aminophenyl)-6-methylbenzothiazole (APMBT) or 2-amino-6-methylbenzothiazole (AMBT).

30

164. The method according to claim 161 wherein the effective amount of the inhibitor ranges from about 1 micromolar to about 1 millimolar in the reaction mixture.

165. The method according to claim 161 wherein the effective amount of the inhibitor ranges from about 1 micromolar and about 500 micromolar in the reaction mixture.

5 166. The method according to claim 161 wherein the effective amount of the inhibitor ranges from about 10 micromolar to about 200 micromolar in the reaction mixture.

10 167. The method according to claim 161 wherein the effective amount of the inhibitor ranges is about 100 micromolar in the reaction mixture.